Under the Microscope

Immune Disorders and Susceptibility to Neoplasms

Om Prakash, PhD; Javed Gill, MD; Gist Farr, MD

Department of Pathology and Laboratory of Molecular Oncology, Division of Research, Ochsner Clinic Foundation, New Orleans, LA

The leading hypothesis that proper immune surveillance prevents the growth of neoplastic cells first emerged at the end of the 19th century, soon after it was recognized that immunity plays an important role in the defense against infection. Subsequent studies by several investigators based on this hypothesis led to the recognition that immunologic surveillance is a common link between allograft acceptance and malignancies in organ transplant recipients. While the degree of immunodeficiency dictates the occurrence of allograft acceptance, it also dictates the occurrence of malignancies.

Over the last 2 decades, there has been a rapid increase in our understanding of the immune system and susceptibility to cancers. It is now clear from the epidemiological studies that the incidence of cancer is significantly higher in populations that are immunocompromised or suffer immune dysregulation. Predominant among these populations are human immunodeficiency virus (HIV)-infected individuals, organ transplant recipients, and persons with genetic immunodeficiency. However, individuals with these conditions do not become susceptible to several common human cancers such as breast, prostate, lung, colon, ovarian, and cervical cancer. Instead, there is a many-fold increase in cancers associated with oncogenic viral infections due to these patients' incompetent immune systems, which cannot suppress the latent infections or fight new infections. Although several malignancies show a rising trend in immunocompromised individuals compared with the general population, two malignancies, Kaposi's sarcoma (KS) and B-cell non-Hodgkin's lymphoma (NHL), etiologically linked to human herpesvirus type 8 (HHV-8) and Epstein-Barr virus (EBV) infections, respectively, have a remarkably high incidence in immunocompromised hosts. This brief overview focuses on our current understanding of the pathogenesis of these two malignancies.

Kaposi's Sarcoma

Among all cancers, KS is one in which the role of immunosuppression is clearly visible. Organ transplant recipients and patients on immunosuppressive therapy are 400 to 500 times more susceptible to develop KS (known as post-transplant KS or iatrogenic KS) than the control population (1). Lesions often disappear when the immunosuppressive therapy is discontinued, suggesting that KS may arise as a consequence of immune dysfunction. However, a direct role of immune dysregulation is controversial since, in some severely immunocompromised patients, KS lesions regress spontaneously (2). Some patients with endemic African-type KS show no detectable immune dysfunction (3). On the other hand, increased risk of KS in persons with genetic immunodeficiency is virtually nonexistent.

The overall risk of KS in patients seropositive for human immunodeficiency virus type 1 (HIV-1), known as epidemic or AIDS-KS, is 20,000 times greater than in the uninfected population in the United States (4). The analysis of the epidemiological data reported to the Centers for Disease Control (CDC) until 1989 indicated that neither HIV-1 infection nor HIV-1-induced immunosuppression was sufficient to explain the markedly high prevalence of KS in HIV-1-infected individuals. There were differences in the incidence of KS within various HIV transmission groups, with the highest prevalence (21%) being found in homosexual or bisexual men and less commonly in those acquiring HIV through heterosexual contact. KS was also found to be more common in women with AIDS who had HIV-infected bisexual men as their sexual partners rather than intravenous drug users. This epidemiological association between KS and AIDS suggested that in addition to immunosuppression the other major risk factor might be a sexually transmissible agent.

Identity of the Transmissible Agent

Although in earlier studies a number of viruses were implicated in various epidemiological forms of KS, including cytomegalovirus, human papilloma virus, and EBV, these findings lacked confirmation. Finally in 1994, Chang et al (5) reported the discovery of a new human herpesvirus in AIDS-associated KS that had partial homology to EBV and herspesvirus saimiri, two herpesviruses capable of inducing cancers in their natural hosts. The new herpesvirus, now referred to as human herpesvirus type 8 (HHV-8, also known as

KS-associated herpesvirus or KSHV) has been shown to be involved in the pathogenesis of all forms of KS, including KS in iatrogenically immunosuppressed individuals. Studies show that in individuals who are at risk for KS, the antibody titers to HHV-8 are highly predictive of disease development and progression (2).

What is the role of immunosuppression in KS pathogenesis? Evidence from a number of studies indicates that KS development is linked to two major risk factors, 1) reactivation of HHV-8 infection, and 2) presence of inflammatory cytokines. Although the immunosuppressive state is not required for the reactivation of HHV-8 infection, it is essential for the virus to escape immunological surveillance to prevent clearance of infected cells. Recent studies indicate that HHV-8 utilizes its own immune evasion mechanism that targets major histocompatibility complex (MHC) class 1 proteins, which are known to play a critical role in the defense against intracellular pathogens (2).

Evidence from a number of studies indicates that virus reactivation occurs in response to inflammatory cytokines (IL-1, IL-6, TNF-α, IFN-γ etc.), which ultimately leads to the spread of virus infection in circulating cells and to the dissemination of these infected cells in various tissues. Individuals with all forms of KS, or at risk for KS, generally present with signs of immune system activation characterized by elevated serum levels of inflammatory cytokines. Treatment of AIDS-KS patients with these cytokines leads to an increase in the progression of KS. Laboratory studies have shown that HIV-1 infection can directly lead to the production of inflammatory cytokines through interaction of viral antigens with HIV-1 specific cytotoxic T lymphocytes, monocytes, and B cells. In transplant patients, allogenic stimulation could provide continuous stimulus for immune activation and inflammatory cytokine production, and consequently, increase the risk of KS. Evidence indicates that inflammatory cytokines participate in the development of KS in many different ways (2). They promote the proliferation and angiogenic properties of KS cells and induce normal endothelial cells to acquire spindle cell morphology through the induction of angiogenic factors and chemokines. Inflammatory cytokines also induce the activation of HHV-8 in latently infected circulating cells that leads to virus spreading into tissues and is likely facilitated by the immunodeficient state of the host, which allows infected cells to escape immune responses. Moreover, HHV-8 encodes cellular homologues of cytokine and chemokine genes, which may further provide a cytokine-rich environment favorable for the development and progression of KS.

The high frequency and aggressiveness of KS in AIDS patients is in part due to the cellular effects of the HIV-1 transactivator Tat protein. The HIV-1 Tat is actively released into the extracellular medium during HIV-1 infection of T cells. Extracellular Tat can induce the proliferation, migration, and invasion of KS cells and normal endothelial cells that have been exposed to inflammatory cytokines present in the conditioned medium from activated T cells. A recent study from our laboratory has shown that human KS cells inoculated in Tat transgenic mice develop into significantly larger tumors compared with the tumors in nontransgenic mice or in mice expressing truncated Tat protein, suggesting that extracellular Tat can contribute to the pathogenesis of AIDS-KS (6).

An unusual feature of KS is that, at least in the early stages, it does not present as a true neoplasm but rather as a benign proliferative lesion that can regress upon withdrawal of immunosuppressive therapies in transplant patients, or regress spontaneously (in some instances) in severely immunocompromised individuals. However, late-stage KS lesions may sometimes develop as a true clonal malignancy resulting from a single or multiple genetic alterations. Since epidemiologic data indicate that KSHV has a crucial role in the pathogenesis of KS, it seems likely that in the initial stages virus infection may contribute to the production of inflammatory cytokines for the development of early-stage lesions, but eventually may contribute to late-stage transformed phenotype through its own transforming ability or through its cooperation with the host oncogenes. However, the precise role of KSHV in malignant transformation in KS remains to be elucidated.

Non-Hodgkin's Lymphoma

All the three immunodeficiency states share a consistently high risk of NHL. According to a 1998 US study, the relative risk of developing NHL in HIV-infected individuals between the ages of 25-44 is 123 times greater than in the general population (7). The renal and heart transplant recipients show 20- and 120-fold increases in NHL, respectively, during the first year (8). Since heart transplant recipients receive more aggressive treatment during the early post-transplant period than kidney recipients, the cumulative incidence of NHL after 5 years is five times higher after heart transplant than after kidney transplant. This supports the widespread belief that risk of NHL parallels the strength of immunosuppressive treatment. NHL also accounts for one-half of all the malignancies in patients with primary immunodeficiency syndrome and has a very high incidence (>70%) in Wiskott-Aldrich syndrome and severe combined immunodeficiency (9). Most of these cases occur in children with median age of 7.1 years at diagnosis. A high male predominance reflects the contribution of X chromosome-linked immunological defects.

Post-transplant lymphoproliferative disorders (PTLDs) are a heterogenous group of lymphomas in transplant patients who receive immunosuppressive therapies. Most of these (86%) are of B-cell origin; only a few cases are of T cell origin. The clinical presentation of PTLDs ranges from polyclonal hyperplasias to monoclonal malignant lymphomas. Based on their morphological and molecular characteristics, PTLDs have been divided into three distinct categories: 1) Plasmacytic hyperplasia, which is usually polyclonal; 2) polymorphic B-cell hyperplasia and polymorphic B-cell lymphoma, which are usually monoclonal; and 3) immunoblastic lymphoma or multiple myeloma, which are monoclonal and contain alterations of one or more oncogene or tumor suppressor genes (ras, C-myc, p53, or others) (10). The third category may also contain monomorphic PTLDs, including large B-cell lymphomas

and Burkitt's or Burkitt's-like lymphomas. Based on a number of studies of heart and kidney transplant recipients, it is generally believed that the incidence of PTLDs is directly related to the strength of immunosuppression treatment. Thus, the first line of treatment to resolve PTLD is to reduce immunosuppression. However, the likelihood of response to this treatment is unpredictable and decreases significantly especially when viewed in terms of duration after transplantation. In some cases reduction of immunosuppression does not promise permanent resolution and even predisposes the patient to rejection of the allograft. Thus, there has always been a need for markers predictive of whether a given PTLD will be responsive to reduced immunosuppression. To explore this possibility, Cesarman et al (11) examined 57 PTLDs from 36 solid organ transplant patients for BCL-6 gene mutations. The BCL-6 gene was selected as the marker because structural alterations in the 5' noncoding region of the BCL-6 gene have been shown in all cases of diffuse large-cell lymphoma and in the majority of cases of follicular and AIDS-related NHLs. BCL-6 gene mutations were found in 43% of polymorphic lesions and in 90% of PTLDs diagnosed as NHL or multiple myeloma. The lesions showing BCL-6 mutations also showed lack of response to reduced immunosuppression, suggesting that BCL-6 gene is a useful marker to determine therapeutic strategies.

EBV Association

EBV is linked to virtually all PTLDs. There is evidence that viral load in the blood correlates with the emergence of PTLD in recipients of solid organ and bone marrow transplants (12). Adoptive transfer of EBV-specific cytotoxic T-lymphocytes can control PTLDs in some patients (13,14), suggesting that cellular immunity against EBV plays a vital role in controlling the EBV-induced lymphoproliferative disorders. This is also consistent with the reports that the frequency of developing PTLD is significantly higher in patients who receive T-cell-depleted stem cell allografts and those who are seronegative prior to transplantation. Seronegativity to EBV is a major risk factor among pediatric transplant recipients.

Although EBV-negative tumors are uncommon in transplant recipients, in cohorts of people with HIV, EBV is linked to about 30% of Burkitt's lymphoma, 80% of immunoblastic lymphomas, and virtually all primary central nervous system NHLs (15). A small percentage of NHLs are primary effusion lymphomas (PEL) and are associated with HHV-8 infection (16). Evidence suggests that in addition to immunodeficiency, people with AIDS have other immunoregulatory dysfunctions that favor the development of lymphomas through chronic stimulation of B cells. For example, HIV-1 infected individuals who develop NHL show elevated levels of CD23, a B-cell differentiation marker, and interleukin-6 (IL-6), a B-cell stimulatory cytokine, compared with those who did not develop NHL.

Male patients with X-linked lymphoproliferative syndrome (XLP) have an inherited susceptibility to EBV infection that predisposes them to a variety of diseases, including fatal infectious mononucleosis, acquired hypogammaglobulinemia, or a non-Hodgkin's malignant lymphoma. Affected boys are usually healthy until they become infected with EBV. The risk of developing lymphomas in these patients is 200 times greater than that in the general population (17).

Although EBV is an important factor in XLP, only about one-half of the lymphomas in primary immunodeficiency diseases are EBV-positive (9). It is very likely that the susceptibility of developing lymphomas in other primary immunodeficiency diseases is more closely linked to certain other uncharacterized defects in the immune system. For example, patients with X-linked hyperimmunoglobulin M syndrome, common variable immunodeficiency disease, and selective immunoglobulin deficiency present clinically with enlarged lymph nodes and splenomegaly.

Other Risk Factors

Although immunosuppression and primary EBV infection represent two major risk factors for PTLDs, several other factors contribute to a highly variable clinical picture. One major factor that deserves consideration is the fact that the nature of the immunosuppressive regimen used can significantly influence the risk of PTLD. A comparison of cyclosporin-based and azathioprin/cyclophosphamide-based regimens revealed that 25% of patients developed lymphomas 15 months after transplantation in the former group versus only 11% in the latter after 48 months of transplantation (18). The tumor-promoting effect of cyclosporin is further supported by laboratory studies showing that it can promote tumor growth in immunodeficient SCID beige mice and promote EBV-induced transformation of human B cells (19,20). Clinical trials have also implicated azathioprine and its metabolite 6-mercaptopurine in the development of NHL in solid organ transplant patients and in patients undergoing treatment for rheumatoid arthritis (21,22). Other immunosuppressants, FK506 and monoclonal antibody OKT3 targeted against human CD3 T lymphocytes, are also associated with a high risk of PTLD (23, 24). PTLD developed in 1.3% of the patients who did not receive OKT3 compared with 11.4% who received the drug. The increase in risk was also dose-dependent. Continuous use of immunosuppressive and cytotoxic drugs may also directly damage DNA and increase susceptibility to neoplasms. For example, alkylating agents have been strongly implicated in causing leukemias and bladder carcinomas (25). Immunosuppressive and cytotoxic drugs may also increase susceptibility to ionizing radiation and ultraviolet rays in causing leukemias and skin cancers.

The host's genetic factors may influence susceptibility to cancers even in the absence of primary immunodeficiency syndromes in several other ways, for example, by altering the immune response to virus infection or by altering the function or expression of certain growth factor genes. IL-6 is an important cytokine for the growth of

Table. Risk of Kaposi's sarcoma and non-Hodgkin's lymphoma in an immunodeficient state.

Cancer	Relative Risk (Fold)			Infectious Agent
	HIV-1 Infection	Transplant Recipients	Primary Immunodeficiency	
Kaposi's Sarcoma	20,000 (4)	400-500 (1)		Human herpes virus type 8
Non-Hodgkin's Lymphoma	123 (7)	20 (renal) (8) 120 (cardiac) (8)	200 (X-linked lymphoproliferative disease) (17)	Epstein-Barr virus

AIDS-KS cells. There is evidence that IL-6 promoter polymorphism plays an important role in HHV-8 infection and in the risk of KS in HIV-1-infected individuals (26). Homozygotes with IL-6 allele G, which is associated with increased IL-6 production, are at increased risk of HHV-8 infection and development of KS compared with allele C homozygotes. Elevated levels of IL-6 are also associated with increased risk of NHL development in HIV-1-infected individuals. EBV-associated NHL patients with primary and secondary immunodeficiencies also show elevated levels of IL-4 with concomitant decrease in levels of α-interferon, a cytokine responsible for IL-4-dependent B-cell proliferation. Thus, it is clear from our existing knowledge that malignancies in an immunosuppressed state could arise from a complex interplay of multiple factors.

Concluding Remarks and Enigmas

It is clear from the epidemiological studies that immunodeficiency, whether genetic, drug-induced, or related to HIV-infection, significantly increases the risk of KS and NHL (Table). Although at first glance the two major risk factors, immunosuppression and oncogenic viruses (HHV-8 and EBV), appear to be straightforward, several questions still remain. For example, the incidence of KS in HIV-1-infected individuals is exceptionally high, whereas KS is less frequent in HIV-2 infection despite a high prevalence of HHV-8 infection and severity of immunodeficiency in both populations. There could be several possibilities; one that has been considered by some investigators is that the Arg-Gly-Asp (RGD) domain that mediates the growth and migration of endothelial and KS cells is not present in HIV-2 Tat. This is also supported by our own recent study showing that human KS cell-derived tumors grow at a significantly higher rate in Tat transgenic mice compared with mice transgenic for RGD-deleted Tat (6).

The widespread use of highly active antiretroviral therapy (HAART) has dramatically decreased the incidence of KS in HIV-infected individuals (27). Although the underlying mechanisms remain to be understood, it appears that HAART may function through inhibition of HIV replication that decreases the pool of growth-promoting cytokines and through partial restoration of the immune system. HAART may also exert direct or indirect effect on HHV-8. Evidence indicates that patients on HAART show decreases in HHV-8 burden in their peripheral blood cells (28). In contrast to its effectiveness in AIDS-KS, a number of epidemiological reports suggest that HAART has a less favorable effect on AIDS-associated lymphomas (15,27). Among the subset of NHL, although a decline in systemic large cell immunoblastic lymphoma and primary nervous system lymphoma was observed, there was no statistically significant change in the incidence of other lymphomas including Hodgkin's lymphoma (27). Since only one-half of NHLs in people with HIV-infection are EBV positive, the data suggest that control of HIV infection has no direct effect on the reduction of lymphomas. Thus, the etiology of EBV-negative lymphomas in HIV infection remains to be investigated. It appears from an earlier study that B cell stimulation in the early phase of HIV infection might be a predisposing factor for AIDS-related NHL even in cases where EBV is not the etiologic factor (29). It is intriguing that HAART decreases the incidence of only those NHLs in HIV infection that are EBV positive, suggesting that HAART can decrease EBV infectivity. However, a recent study suggests that HAART could be a risk factor for lymphoma development in HIV-1 and EBV-coinfected patients, especially those who show significant improvement in their immune status but lack HIV-1 suppression during therapy (30). Thus, there is a need to carefully evaluate the benefits of HAART in HIV-1 and EBV-coinfected patients when HIV-1 replication is not significantly suppressed, even though they show an increase in CD4 cell counts.

An interesting feature of KS and NHL is their ability to undergo spontaneous remission when the immune status returns to normal. Now we know that regression is more likely to occur when the lesion is hyperplastic rather than truly neoplastic. Hopefully, new findings that HHV-8 encodes transforming proteins and that regression- resistant PTLDs result from genetic alterations will lead to the basic understanding of the disease process and prognostic factors in all the three immunodeficiency states. It is also intriguing that individuals with XLP syndrome alone develop susceptibility to EBV infection but not with other primary immunodeficiency syndromes that are also risk factors for NHL. Further investigations toward understanding host factors and viral factors in these immunodeficiency states will provide novel insights into the pathogenesis and management of KS and NHL.

Acknowledgements

The authors wish to thank Janet Buroff, MD, and John Cole, MD for their helpful suggestions.

References

1. Penn I. Neoplasia: an example of plasticity of the immune response. Transplant Proc 1996; 28:2089-2093.
2. Ensoli B, Sturzl M, Monini P. Reactivation and role of HHV-8 in Kaposi's sarcoma initiation. Adv Cancer Res 2001; 81:161-200.
3. Kestens L, Melbye M, Biggar RJ, et al. Endemic African Kaposi's sarcoma is not associated with immunodeficiency. Int J Cancer 1985; 36:49-54.
4. Beral V, Peterman TA, Berkelman RL, et al. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet 1990; 335:123-128.
5. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994; 266:1865-1869.
6. Prakash O, Tang ZY, He YE, et al. Human Kaposi's sarcoma cell-mediated tumorigenesis in human immunodeficiency type 1 tat-expressing transgenic mice. J Natl Cancer Inst 2000; 92:721-728.
7. Selik RM, Rabkin CS. Cancer death rates associated with human immunodeficiency virus infection in the United States. J Natl Cancer Inst 1998; 90:1300-1302.
8. Swinnen LJ. Diagnosis and treatment of transplant-related lymphoma. Ann Oncol 2000; 11 Suppl 1:45-48.
9. Filipovich AH, Mathur A, Kamat D, et al. Primary immunodeficiencies: genetic risk factors for lymphoma. Cancer Res 1992; 52(Suppl 19):5465s-5467s.
10. Knowles DM, Cesarman E, Chadburn A, et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood 1995; 85:552-565.
11. Cesarman E, Chadburn A, Liu YF, et al. BCL-6 gene mutations in posttransplantation lymphoproliferative disorders predict response to therapy and clinical outcome. Blood 1998; 92:2294-2302.
12. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. Blood 1994; 84:972-984.
13. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 1995; 345:9-13.
14. Dazzi F, Goldman JM. Adoptive immunotherapy following allogeneic bone marrow transplantation. Annu Rev Med 1998; 49:329-340.
15. Grulich AE. AIDS-associated non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 1999; 21 (Suppl 1):S27-S30.
16. Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995; 332:1186-1191.
17. Grierson H, Purtilo DT. Epstein-Barr virus infections in males with the X-linked lymphoproliferative syndrome. Ann Intern Med 1987; 106:538-545.
18. Penn I. Cancers complicating organ transplantation. N Engl J Med 1990;323: 1767-1769.
19. Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999; 397:530-534.
21. Wilkinson AH, Smith JL, Hunsicker LG, et al. Increased frequency of posttransplant lymphomas in patients treated with cyclosporine, azathioprine, and prednisone. Transplantation 1989; 47:293-296.
22. Silman AJ, Petrie J, Hazleman B, et al. Lymphoproliferative cancer and other malignancy in patients with rheumatoid arthritis treated with azathioprine: a 20-year follow up study. Ann Rheum Dis 1988; 47:988-992.
23. Sokal EM, Antunes H, Beguin C, et al. Early signs and risk factors for the increased incidence of Epstein-Barr virus-related posttransplant lymphoproliferative diseases in pediatric liver transplant recipients treated with tacrolimus. Transplantation 1997; 64:1438-1442.
24. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990; 323:1723-1728.
25. Penn I. De novo malignances in pediatric organ transplant recipients. Pediatr Transplant 1998; 2:56-63.
26. Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 2000; 96:2562-2567.
27. International Collaboration on HIV and Cancer. Highly active antiretroviral therapy and incidence of cancer in human immunodeficiency virus-infected adults. J Natl Cancer Inst 2000; 92: 1823-1830.
28. Wit FW, Sol CJ, Renwick N, et al. Regression of AIDS-related Kaposi's sarcoma associated with clearance of human herpesvirus-8 from peripheral blood mononuclear cells following initiation of antiretroviral therapy. AIDS 1998; 12:218-219.
29. Przybylski GK, Goldman J, Ng VL, et al. Evidence for early B-cell activation preceding the development of Epstein-Barr virus-negative acquired immunodeficiency syndrome-related lymphoma. Blood 1996; 88:4620-4629.
30. Righetti E, Ballon G, Ometto L, et al. Dynamics of Epstein-Barr virus in HIV-1-infected subjects on highly active antiretroviral therapy. AIDS 2002; 16:63-73.

Dr. Prakash is the Head of Ochsner's Molecular Oncology Laboratory and an Associate Professor (conjoint) in the Department of Microbiology, Immunology & Parisitology at the Louisiana State University Health Sciences Center.